The Pending Claims

The present invention relates to a method for detecting a target nucleic acid sequence which may employ both an amplification procedure and a detection procedure. In the amplification procedure, an amplification sequence of the target is amplified using at least two pairs of contiguously hybridizing amplification probes which are ligated to form complementary amplification product. It is preferred to use three or more pairs of amplification probes. In the preferred embodiment, a detection procedure may be used to distinguish the majority of spurious blunt-end ligated amplification by-product (which is coincidentally formed in solution without benefit of alignment on a template) from correctly assembled amplification product using at least two detection probes. Each detection probe is complementary to a portion of each of a different combination of two adjacently situated amplification probe segments of the amplification product. The correctly assembled amplification product serves as a template such that the detection probes hybridize to the amplification product in a contiguous manner, and may subsequently be ligated substantially only if brought together by correctly assembled amplification product.

Claims 1-5 are directed to the amplification procedure of the present invention, with claims 6-13 covering the detection procedure. The combined amplification/detection procedure is covered in claims 14-18. Claims 19 and 20 are directed to reagents for the amplification and detection procedures, respectively, and claim 21 is directed to a kit, or set of reagents, for use in the combined amplification/detection procedure.

The References Relied on by the Examiner

Applicants are especially concerned that the arguments raised by Applicants with respect to the 35 USC §103 rejections were not addressed. The Examiner has rejected all of claims 1-21 of the present application under 35 USC §103 on the basis of three

references, namely Whiteley *et al*, Mullis *et al*, and Palva *et al*. Applicants have previously provided (in their amendment filed October 27, 1989) a brief description of the references relied on by the Examiner. Applicants have taken the opportunity to highlight herein some of the issues previously raised by Applicants which were not addressed in the most recent office action.

Obviousness Rejection of Claims 19-21

The Examiner rejected reagent and kit claims 19-21 as being obvious over Whiteley *et al*, alleging that it would have been routine to synthesize complementary strands of the Whiteley *et al* contiguous detection probes. Applicants have previously raised the argument that the present invention employs pairs of probes, and that simply providing complementary strands in the same reagent with the Whiteley *et al* contiguous probes is not the same as providing pairs of probes, to achieve amplification of a target. Claims 19 and 21 specifically recite pairs of amplification probes.

Obviousness Rejection of Claims 1-5

The Examiner rejected amplification claims 1-5 on the grounds that the contiguous probes of Whiteley *et al*, when combined with the teachings of Mullis *et al*, can be repeated through "several cycles" to achieve amplification as claimed by Applicants. Applicants have, however, previously pointed out that there is no teaching, or even suggestion, in either Mullis *et al* or Whiteley *et al*, that the Whiteley *et al* procedure can be adapted in any way to achieve amplification of a target sequence. Mullis *et al* disclose only one method for amplifying a target sequence, with no suggestions for other methods of achieving amplification. The Whiteley *et al* reference, on the other hand, is simply an example of the ligation of two contiguous probes in order to discriminate correctly bound lengthened detection probe (ligated product) from spuriously bound shorter detection probe. There is no suggestion in either reference as to how the probes of Whiteley *et al* should be selected and aligned with respect to the

target sequence in order to achieve amplification, and no teaching with respect to the use of pairs of probes.

It is particularly troubling to Applicants that the Examiner has repeated his earlier objection with respect to Applicants' preferred use of three or more pairs of probes, alleging that it "would have been further obvious to include more probes in the Whiteley-Mullis procedure for the lower probability of unspecific probe hybridization as Whiteley *et al* disclose." As Applicants previously argued, increasing the number of probes in Whiteley *et al* would only result in decreased sensitivity (by adding the requirement for an additional ligation event) and would not affect specificity at all. Applicants believe it is important to reiterate that increasing the number of pairs of amplification probes in Applicants' amplification procedure results in increased production of undesired spurious blunt-end amplification by-product, which is only addressed, in an unobvious way, by Applicants' detection system.

Obviousness Rejection of Claims 6-13

The Examiner has repeated his rejection of Applicants' detection claims, 6-13, as being unpatentable over Whiteley *et al* in view of Palva *et al.* Applicants have difficulty, however, appreciating the contribution(s) of Palva *et al* to the present invention. The Palva *et al* invention makes use of "at least two series" of alternating probes which are situated "close to but not adjacent to one another." The multiple capture probes comprise one "series", with the detection probes comprising the other "series", such that the two series of probes "alternate" in position along the target in the following manner: "capture-detection-capture-detection-etc.". The probes are expressly designed not to be contiguous. While the multiple capture probes can be ligated together, this ligation is suggested only for convenience in attaching the capture probes to an immobilizing support. There is nothing in Palva *et al* which remotely suggests how to use contiguous detection probes to detect correctly assembled amplification product. By contrast, the present specification teaches

how to select contiguous detection probes to span amplification probe segment junctions of amplification product in order to discriminate correctly assembled amplification product from incorrectly assembled by-product.

Most importantly, the Examiner has completely failed to address the Backman *et al* reference (European Patent Application No. 320,308) cited by Applicants, which also discloses an amplification system using multiple probes to form amplification product. Backman *et al* recognize the same blunt-end ligation problem as recognized by Applicants, but approach the problem by attempting to prevent or diminish the amount of spurious blunt-end ligated amplification by-product by manipulating the terminal, or end probes of a series of amplification probes. This approach is not effective, however, in Applicants' preferred amplification procedure because the middle pair(s) of probes cannot be manipulated in the manner taught by Backman *et al.* For example, in an amplification procedure employing four pairs of amplification probes, one-half of the total amplification probes cannot be manipulated in accordance with the Backman *et al* teachings. Applicants' approach is to overcome, rather than merely avoid, the blunt-end problem. This enables Applicants to make use of increasing numbers of pairs of probes to improve sensitivity. (The only other solution presented by Backman *et al* for alleviating the blunt-end ligation problem is to limit the number of amplification cycles. This, in turn, limits the sensitivity of the amplification procedure, yielding a result which is self-defeating.)

Obviousness Rejection of Claims 14-18

In light of Backman *et al*, and Applicants' unobvious approach to the blunt-end problem, it is even more attenuated that the Examiner has again summarily rejected amplification/detection claims 14-18 as being unpatentable over Whiteley *et al* in view of both Mullis *et al* and Palva *et al.* It is worth repeating that Applicants' ability to discriminate between correctly assembled and